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THE EFFECT OF DIESEL EXHAUST ON SPERM-SHAPE
ABNORMALITIES IN MICE

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THE EFFECT OF DIESEL EXHAUST ON SPERM-SHAPE

ABNORMALITIES IN MICE

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ABSTRACT

The sperm-shape abnormality bioassay in mice was used to determine whether chemical mutagens in diesel exhaust reach the testes. Strain A male mice (30 per group from 4 to 6 weeks of age) were exposed for 31 or 39 weeks to either diesel exhaust or clean air. After exposure, Eosin y-stained, air-dried smears of cauda epididymal sperm were scored for changes in sperm-head abnormalities in three different laboratories. There was no difference in the proportion of abnormally shaped sperm in controls and mice exposed to diesel exhaust.

Diesel exhaust emissions contain thousands of organic chemicals, including known carcinogens and mutagens. Because of the high particle concentration of diesel exhaust a large proportion of the organics, especially the polycyclic aromatic hydrocarbons, are

adsorbed onto particles (1). For genotoxic agents in diesel exhaust to affect the mammalian testes they must be deposited in the lungs, eluted from the particles, and distributed in the body so that active metabolites can reach the testes.

The murine sperm-shape abnormality bioassay provides a way of assessing possible spermatotoxic and genotoxic effects of chemical agents in vivo (2). Studies in mice have shown that a) sperm-head shape is under genetic control, b) chemical induction of sperm abnormalities is correlated with germ-cell mutagenicity, and c) mutations associated with induced sperm abnormalities can be inherited in the offspring of treated mice (3). Several other factors, such as x-rays, heat, and severe fevers involving the testes can also induce sperm-head abnormalities (3). Besides identifying testicular toxins and mutagens the sperm abnormality assay may also be an effective indicator for carcinogens. For example, sixty five (65%) percent of 38 known carcinogens tested by this bioassay showed positive results, a correlation very similar to that found using the Salmonella bioassay, which is associated with carcinogenic potential (4).

In this communication, we report the effect of inhalation exposure to diesel exhaust on the murine testes using the sperm-head abnormality assay.

METHODS AND MATERIALS

Male mice (strain A/Strong, Strong Research Foundation, Calif.) at 4 to 6 weeks of age were divided into 4 groups of 30 mice each and exposed to either clean air or diesel exhaust emissions for 31 or 39 weeks. Thirty-one and 39 weeks were chosen because they represent approximately 6 and 8 complete spermatogenic durations. This experiment design permitted 2 independent assessments of the effects of diesel. The diesel exhaust engine and exposure conditions at the U.S. Environmental Protection Agency, Health Effects Research Laboratory facilities in Cincinnati, Ohio, are described elsewhere by Hinners (5). Briefly, a 6-cylinder Nissan diesel engine was run on the Federal Short Cycle and animals were exposed to exhaust for 8 hours daily at 6 mg/m³ particles concentration using a 1:18 dilution.

At the end of the exposure interval, the mice were sacrificed by cervical dislocation, the cauda epididymides excised, and minced in 4 ml of phosphate buffered physiological saline. The suspension from each mouse was mixed by repeated pipetting and filtered through 74 μ m nylon mesh. An aliquot was mixed (10:1) with 1% Eosin-Y prepared in water and incubated for 30 min. Smears were made, air-dried, and mounted with Permount. For each mouse in the control and exposed groups receiving 31 week exposures, 500 sperm were examined in each of three different laboratories. The control and exposed mice receiving 39 week exposures were examined at only one of these laboratories. Examples of normal and abnormal sperm shapes are presented in Figure 1.

RESULTS AND DISCUSSION

The effect of diesel exhaust emission on sperm shape is summarized in Table 1. Although there were some differences in the scoring criteria used in the 3 laboratories, none of the laboratories detected a difference in the percentage of abnormally shaped sperm in mice exposed for 31 weeks to diesel exhaust when compared to clean air controls. Laboratory III scored an additional 2 groups of 30 mice each, that were exposed for 39 weeks to either clean air or diesel exhaust. As shown in Table I, the percentages obtained for these animals were not statistically different from the values obtained for the 31 week exposure, and also showed no difference between control and exposed mice.

These results show that inhalation exposure to diesel exhaust did not induce a detectable increase in sperm head abnormalities in mice used. The failure to detect an increase suggests that the testes is not affected by inhalation of diesel exhaust. The negative finding, however, may be due in part to the genotype used in this study. Although the A/Strong strain is genetically able to activate and metabolize hydrocarbons, it has a very high spontaneous rate of sperm shape abnormalities in unexposed males (approximately 27%, see Lab III, Table I) when compared to the 1 to 2% found in B6C3F1 hybrid males which are normally used in this bioassay (6). This high background rate and the unusually high variance in the unexposed mice could have masked any small positive effect. The failure to detect increases in sperm shape abnormalities might also have been due to 1) the low levels of genotoxic materials in diesel exhaust, 2) the strong adsorption of such chemicals onto the diesel particles which may have prohibited their release in vivo, or 3) the inability of the released materials to be transported from the lung, to be metabolically activated, or to reach the testes.

Further studies are planned to a) identify, characterize, and quantitate the amount of genotoxic agents in diesel exhaust particles and their purified extracts, b) to evaluate the role of particle adsorption, metabolic transport, and activation, and c) to compare the genotoxic activities of purified agents in vitro and in vivo.

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REFERENCES

1. Huisingh, J., R. Bradow, R. Jungers, L. Claxton, R. Zweidinger, S. Tejada, J. Bumgarner, F. Duffield, M. Waters, V.P. Simmon, C. Hare, C. Rodriguez, and L. Snow, 1978. Application of Bioassay to the Characterization of diesel particle emissions. In: Application of short-term bioassays in the fractionation and analyses of complex environmental mixtures, pp. 381-418. EPA publication 600/9-78-027.
2. Wyrobek, A.J. and W.R. Bruce, 1975. Chemical Induction of Sperm abnormalities in mice. Proc. Nat. Acad. Sci. USA, 72:4425-4429.
3. Wyrobek, A.J. and W.R. Bruce, 1978. The induction of sperm-shape abnormalities in mice and humans. IN: Chemical Abnormalities: Principles and Methods for Their Detection, A. Hollaender and F.J. de Serres (Eds.), Vol. 5, 257-284, Plenum Press, N.Y.
4. Heddle, J.A. and W.R. Bruce, 1977. Comparison of tests for mutagenicity or carcinogenicity using assays for sperm abnormalities, formation of micronuclei and mutations in Salmonella. IN: Origin of Human Cancer, H.H. Hiatt, J.D. Watson and J.A. Winsten (Eds.) Vol. 3, 1549-1557, Cold Spr. Harb. Lab., N.Y.
5. Hinners, R.G., J.K. Burkart, M. Malanchuk, and W.D. Wagner, 1980. Facilities for diesel exhaust studies. Proceedings of the International Symposium on Health Effects of Diesel Engine Emission, Dec. 1979.
6. Wyrobek, A.J., 1979. Changes in mammalian sperm morphology after x-ray and chemical exposures. Genetics Suppl. 92: s105-s119.

TABLE I. EFFECT OF DIESEL EXHAUST ON SPERM MORPHOLOGY IN MICE

Treatment	Number of Animals	Percentage of Abnormally Shaped Sperm			
		<u>Lab I^a</u>	<u>Lab II^a</u>	<u>Lab III^a</u>	<u>Lab III^b</u>
Diesel Exhaust	30	12.0 $\pm$ 7.7 ^c	13.9 $\pm$ 7.5	27.7 $\pm$ 9.7	27.1 $\pm$ 8.2
Clean Air	30	9.2 $\pm$ 4.4	14.9 $\pm$ 5.2	27.8 $\pm$ 12.2	27.3 $\pm$ 10.6

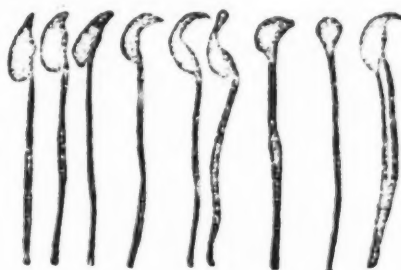
^a31 week exposures^b39 week exposures^cPercent of abnormally shaped sperm, average $\pm$ standard deviation.

Figure 1. Shapes of normal and abnormal sperm from mice.

SPERM - SHAPE ABNORMALITIES



NORMAL



ABNORMAL

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